# Chapter 5

# Discussion

## 5. Discussion

Insects comprise about three-fourths of all species of animals worldwide (Majumder *et al* 2013). Insects have many ecological roles in nature that are mostly benefiting for humans. Edible insects are important natural resource found abundantly in Assam- a biodiversity hotspot of the world. The edible species contain many useful nutrients, including proteins and calories. Some insects besides being used as food were also used in the treatment of certain diseases. *Gryllotalpa africana* has been used as therapeutic food for healing certain childhood diseases and *Nephila* in preparation of folk medicines (Narzari and Sarmah 2015a). The consumption of certain species of insects is seen during festivals, in the form therapeutic and as a component of folk medicine. The far-reaching information presented by the Bodo tribe with regard to the medicinal uses of insects, can convey an extensive general awareness on the usefulness of insects in health and healing. The consequent turns down on availability of insects as a superseding reason, spots a feasible environmental reason for switching of food sources. The indication given by respondents as unfavourable weather and climatic changes for decline in entomophagy is likely to further pessimistically affect local food production thereby endangering global food security (Casabona *et al* 2010). Decline in any food source is an alarmingly stressful situation. Edible insects are in essence gratis foods that will chiefly impact folks who make the most of the resource from need.

Insect foods are preferred for its tastiness as cited by Hanboonsong (2010) in Thailand. Likewise, the Bodos of Assam, India prefer insect owing to its taste. The Bodos are proud of their traditional roots and this may be one rationale for the optimistic view they take of entomophagy. They are therefore seen to be reluctantly involved in buying and selling insects ensuring that the consumers possesses rich knowledge of a reasonably cheap and nutritious food. Assigning a distinct local name for each species consumed for easy communication and distinct traditional methods of cooking and consuming them reflect how dear the food is for the Bodos of Assam.

Insects have evolved with an astounding collection of morphological organization and biological traits. Most terrestrial ecosystems have been colonized by large number of varied insects. Thus, it is unquestionably challenging to write the

biology of each and every insect's ecology and diversity encasing all fields from morphology, taxonomy, phylogeny, genetics, physiology and behavior in a well written and exhilarating way. Taxonomic identification of animal and plant species is often emphasized as a basic prerequisite in many biology lenient studies. Circulating fresh and unique information concerning any organism is unfeasible without the prior verification of its universal scientific name (Randler 2008). For lesser known species this course of action universally is undertaken by professional taxonomists. Many insects undergo metamorphosis and insect consumers mostly harvest edible insects at particular metamorphic stages preferred for consumption. This process becomes more difficult when the case of edible insects is considered particularly when only a single life stage is consumed. This state of affairs is common in identifying edible insects especially species from lepidoptera and odonata which undergo subsequent metamorphic changes that cannot be linked unless the life cycle is restudied. Artificial rearing of wild species to adulthood is a necessity for correct identification of certain species that experiences metamorphosis. Thus by far in this study; the collaboration of taxonomists from Zoological Survey of India (ZSI) Shillong and Kolkata has contributed to the identification of 25 insect species of which seventeen were classified upto species level, two upto genus and six upto order. In the present study, the English terminology for orthopterans was quite confusing as most of them were grasshoppers. Hence grasshoppers are more often collectively referred in many studies. Further, the scientific name and the vernacular name provided here can help in better understanding or identifying the grasshopper species more precisely. Accurate identifications of species consumed can endorse research priority and more attention. Focus should be bestowed on the conservation of the edible insect gene pool.

**5.1 Nutritional values**

Edible insects show potential indications for more in depth examinations into aspects of its nutritional significance. Preliminary investigations demonstrate that edible insects form a high quality food that is safe to consume. References on nutritional values of insects are available from a wide range of scientific disciplines

and a great many of them justify that they are a rich source of quality proteins (Defoliart 1989; Finke *et al* 2012). Other references spot the abundance of other nutrients as carbohydrates, vitamins, minerals and fatty acids in edible insects worldwide and their utilization in human and animal nutrition (Vantomme 2010; Bophimai and Siri 2010; Gbogouri *et al* 2013; Yeo *et al* 2013). Comprehending the health implication of any promoted food and principally if this is a novel food is important. Populations living in plenty as well as in scarcity of food resources have an equal chance of receiving inadequate nutrients from food, the rich may experience this due to negligence of food habits and the poor due to lack of food resources (Melo *et al* 2011). For the maintenance of a healthy physic and mind the first anticipated approach is to consume the standard recommended daily intake level of nutrients (Mutalik *et al* 2011). To ascertain the future food security, search for new food resources involving identification and development of localized ethnic ones continues.

As food, insects form a small part of the bulk of diet among the Bodos. They are prepared in small quantities to be consumed along with rice as curry or fries. The minor quantity of insect food consumed is important for compensating for the general deficiency in animal proteins, fats, and calories among the consumers (De Foliart 1975).

This study on the chemical composition of edible insects demonstrates that the species consumed by the Bodos of Assam, India represent healthy and nutritious food items. Hence, this study confirms those published documents on the nutritional composition of edible ants by other researchers, but it also illustrate that considerable differences in the chemical composition can exist owing to reasons as closely related phylogeny, identical species from different locations, climatic factors and feeding behaviours (Paoletti *et al* 2003; Banjo *et al* 2006; Ekpo and Onigbinde 2007; Agbidye *et al* 2009a; Yhoung-Aree and Viwatpanich 2005; Raksakantong *et al* 2010; Chakraborty *et al* 2016). It is therefore not surprising to find such differences reflected in the insects' nutritional compositions. This study also stresses that the nutritional analyses of the insects are based on individuals collected from

areas in which the local populace made use of them as food. The nutritional values of this locally collected insect species are discussed here accordingly.

### 5.1.1 Proximate Compositions

#### 5.1.1.1 Moisture

The most common and ancient traditional method of food preservation among the Bodos is sun drying. Food sample containing low moisture indicate good shelf life characteristics (Bhulaidok 2010). The moisture content of the insect food studied on dry weight basis ranged from highest (1.67 – 8.87%). Most species indicated a lower moisture content that was lesser compared to that of sun dried Zhejiang ants (12.8%) and corroborated with the moisture values 8.6% of Guizhou sun dried ants mentioned by Bhulaidok (2010). The highest amount of moisture 8.87% in this study was retained in *Parapolybia varia*. Lowest amount of moisture was observed in an aquatic *Odonata* sp. (Garba fangthe) thus indicating its good shelf life. The moisture content of *Macrotermes* sp. 2.91% is comparable to that of *Macrotermes belicosus* 2.82% and *Macrotermes notalensis* 2.98% as described by Banjo *et al* (2006). All hymenopterans analysed for moisture in this study had higher moisture values that was above (6%) and was much higher compared to that of *Apis mellifera* 3.82% as reported by Banjo *et al* (2006). Thus hymenopterans indicated a poor shelf life when compared to the other species. Moreover, aquatic insects as *L. indicus*, *C. tripunctatus* and aquatic Odonata species showed lesser moisture retention and improved shelf life when dried. Moisture percent in orthopterans were seen to be moderate ranging between (3.97 - 6.49%) which was lesser and better in comparison to that of the hymenopterans. Normally the Bodos consume fresh edible insects except when collection becomes excess they are sun dried and stored for future consumption. Moisture percentage in any food is determined to measure the stability and susceptibility of the food to microbial contamination (Scott 1957). Since lower moisture content in food improves the shelf life it may not be favourable for pertaining the quality of insects with high moisture percent like hymenopterans for future use.

### 5.1.1.2 Ash and Minerals

Mineral components in insects are several times higher than many traditional foods and hence these are remarkable nutrients (Siemianowska *et al* 2013). Ash content is indicative of the amount of micronutrients and macronutrients. Ash percentage of the studied species ranging from (0.48 – 8.86) g/100g dry weight indicates that edible insects consumed by the Bodos possesses sufficient amount of minerals which is higher than that of beef, pork, egg and fish from food standards of USDA (2015). Among the hymenopterans *Apis mellifera* is the highest studied hymenoptera for its nutritional benefits. Immature stages of *Apis mellifera* have good food value for man (Hocking and Matsumura 2016). The ash content of *P. olivaceus* 2.60% and *V. affinis* 2.55% is comparable to that of *Apis mellifera* which possesses 2.20% of ash content (Banjo *et al* 2006). *P. varia* showed lower amounts of ash compared to the above mentioned three hymenoptera species. Another hymenoptera seldom reported in Indian entomologic and entomophagic literature is *Oecophylla smaragdina* whose larval stage is preferred for food. A recent study of the nutrient composition on *O. smaragdina* in Arunachal Pradesh of India showed that it comprises 2.586% of ash which is also comparable to that of *P. olivaceus* 2.55% and *P. varia* (Chakravorty *et al* 2016). Findings from this study showed much higher ash percent for *O. smaragdina* (5.16%) in comparision to the findings of Chakravorty *et al* (2016) in *O. smaragdina* consumed by certain tribes in Arunachal Pradesh. The ash content of *V. affinis* (2.55%), *P. olivaceus* (2.6%) and *O. smaragdina* (5.16%) was higher in comparision to that of beef (1.89%), pork (1.43%), chicken (2.32%) and fish (1.46%) (USDA 2015). Though Magnesium levels were found to be lower the Magnesium content of *O. smaragdina* (38.5 mg/100g) was comparable to that of beef (39.8 mg/100g) dry weight. Iron content of *P. varia* (7.5 mg/100g), *P. olivaceus* (7.5 mg/100g) and *O. smaragdina* (10.5 mg/100g) were higher in comparison to conventional food as beef 4.3 mg/100g, pork (1.4 mg/100g) and chicken (2.6 mg/100g) from USDA (2015). Copper content of the studied hymenopterans were found to be higher than that of beef (0.2 mg/100g), pork (0.1 mg/100g), and fish (0.2 mg/100g) from USDA (2015).

The percentage of RDA recorded show that the studied hymenopterans have the potential to supply the required amount of iron, copper and phosphorus for health and nutrition through the diet of the consumers. Macronutrients potassium, sodium, calcium and magnesium requirement levels could not be fully fulfilled but they can be compensated as this species are consumed as part of rice meals. The potentiality of fulfilling the zinc requirement was also low. Higher values for zinc about 74.29% and 63.64% of the recommended dietary intake in both males and females could be fulfilled by *O. smaragdina*. Excess zinc causes anaemia and zinc deficiency in the body can direct to dermatitis so it is important to maintain the standard level of zinc or any other nutrients in our diets.

Among the orthopterans the ash content ranged between (0.48 – 7.93 %) which is comparable to ash content (0.34 – 16.5 %) of orthoptera grasshoppers in Mexico described by Ramos-Elorduy *et al* (2012) and few orthopteran insects described by Bukkens (1997). The study undertaken reveals that the orthopterans consumed by the Bodos are rich sources of iron and copper that can supplement surpassed amounts of iron and copper requirements in adults. The iron and copper content of *E. inflatus, C. robustus, C. rosea and M. elongata elongata* were found to be slightly lower than that of *Oxya hyla hyla* 16.19 and 4.36 mg/100 reported by Ghosh *et al* (2016). *T. portentosus* showed higher values of iron and copper compared to the other species. Macronutrients sodium, calcium and magnesium were present in meager amounts. Although potassium and phosphorus were present in adequate amounts it still could not reach the recommended RDA values for adults. *T. portentosus* had higher ash content of 7.93% compared to beef (1.89%), pork (1.43%) and chicken (2.32%) from USDA (2015) on dry weight basis which indicate its high mineral contents. Among the orthopterans; *T. portentosus* is proven to be the richest source of minerals that can supplement good amount of iron through direct nutrition.

Of the many unambiguous kinds of insects that have been utilized as food, one that is considered most curious is the giant water bug, *Lethocerus,* a hemiptera which, being large in size, is consumed like a lobster (Delong 1960). *Lethocerus indicus* species is consumed as a tasty food by the consumers. It is also known for its

high iron content (Shantibala *et al* 2014). In this study the iron content of *L. indicus* was found to be lower than that of the findings of Shantibala *et al* (2014). Though lower in values it could compensate 212.5% of the RDA values for iron intake in males and 94.44% in females so it can be judged as a good source of adequate amount of iron for nutrition. Aquatic insects hold elevated amount of calcium compared to terrestrial insects (Adeduntan 2005). An elevated amount of calcium was observed in this species compared to the other species in this study. The high level of calcium in *L. indicus* may considerably supplement calcium for the development of teeth and bones in developing children and adolescents. The calcium content of other aquatic insects of order odonata and coleoptera were slightly lower but comparable to the range 24.3- 96 mg/100g described by Adeduntan (2005) for aquatic insects. The iron content of all the aquatic insects were within the range 13 – 17 mg/100g. The zinc content was comparatively lower in all the species. Copper was detected in trace amounts but the values detected surpassed the RDA requirements in both males and females so judging this condition the studied species can be predicted as a rich source of copper supplement in human nutrition. *Macrotermes* sp. are the most studied species for their nutritional value and their consumption as food has been documented in a wide range of literature worldwide (Ajayi 2012; Afiukwa *et al* 2013; Alamu *et al* 2013; Banjo *et al* 2006; Chakraborty *et al* 2016). The ash content of termite *Macrotermes* sp. (1.65%) was similar to that described by Banjo (2006). The phosphorus content was found to be higher and iron content lesser than that observed by Banjo (2006) but was found to be higher compared to odontotermes in the study by Chakraborty *et al* (2016). A still higher value of iron (47.5 mg/100g) was observed in *Nephila* sp. which belongs to a rarely studied order in nutritional grounds. *Lepidoptera* sp. (Gunjet) revealed higher iron values 16.5 mg/100g compared to other lepidopteran insects such as *Bombyx mori* which is 1.8 mg/100g (Dunkei 1996) and *Cirina forda* 5.34 mg/100g (Omotoso 2006). Insects have proved to be a better source of iron compared to other conventional foods as red meats (Williams 2007). Most of the species show higher amounts of phosphorus, potassium and sodium than beef, pork and chicken. *L. indicus* had higher amount of calcium and magnesium compared to the other species

and food standards. All the species had higher iron values than that of beef, pork, and fish from food standard reference of USDA (2015). Higher values of copper were observed in *Lepidoptera* sp. (Gunjet) than the other species.

Minerals are an integral part of many life processes. Most edible insects contain higher amounts of iron than beef (Bukkens 2005). The values of copper, potassium and calcium were higher than beef in most of the species. Fe and Zn are the minerals that represent core public health problems, particularly in developing countries, where often mineral deficient foods are consumed by children as well as adults especially women (Michaelsen *et al* 2009). Iron supplementation in infants show benefits of iron on motor and social emotional outcomes and improves short-term cognitive outcomes in preschool – aged children (Walker *et al* 2007). Species lacking sufficient amount of calcium, magnesium, potassium, calcium and can serve as providers of micronutrients when added to cereals or other plant products. Thus about 100g of edible insects consumed can provide sizeable amount of recommended daily intake of iron, copper, zinc, potassium, sodium, magnesium, calcium and phosphorus to the consumers.

**5.1.1.3 Proteins and Amino Acids**

Edible insects are good sources of proteins. The analysis of proteins in the samples investigated showed ranges between lowest 30.25% to the highest 84.56%. Hymenoptera species are best and elaborately studied for their ecological services, honey and painful sting (Huber 2009; Hunt *et al* 1998). Contrary to it even though the larvae of many Hymenopterans are consumed as food, studies related to their nutritional value is infrequent. Study on the proximate composition on five species of hymenopterans consumed as food by the Bodos of Assam reveals that it possesses protein ranges between 50.13 – 53.63% on dry weight basis which is higher than that of *Apis mellifera* 21% (Banjo *et al* 2006) and was within the range 28 – 81% dry weight for order Hymenoptera (Ramos-Elorduy *et al* 1997). All hymenopterans analysed for protein had protein content above 50% the highest was observed in *P. varia* (53.63%). The protein content of *P. Olivaceus* (51%) were comparable to that of *polybia* sp. larvae (51%) (Ramos-Elorduy *et al* 1984). The protein content of *O. smaragdina* (52.13%) was higher than that of sundried Zhejiang (31.5g/100g) and

Guizhou 41.5 g/100g ants of China (Bhulaidok *et al* 2010) but was lower than that observed in *O. smaragdina* (55.27%) in Arunachal Pradesh, India by Chakraborty *et al* (2016) and Chicatana ant 66.00% (Melo *et al* 2011). Species from order orthoptera showed protein content ranges between 30.25 – 78.31% and the range corroborated with the range of protein content for orthopterans 56 – 77%. The highest percent of protein 84.56% was recorded in a terrestrial species *Nephila* sp. from order Araenae which was higher than the protein value (81%) observed by Ramos-Elorduy *et al* (1997) in investigating the protein content of seventy-eight species of edible insects. The protein value of *L. indicus* (67.31 g/100g) and *C. tripunctatus* (59.00 g/100g) was found to much higher than that of *L. indicus* (22.67 g/100g) and *C. tripunctatus* (22.64 g/100g) observed by Shantibala *et al* (2014) in aquatic insects of Manipur, India. Even though the protein content 39.44% of *Macrotermes* sp. was found to be lower than most of those studied species it closely resembled to that of *Macrotermes bellicosus* (38.36%) (Ekpo *et al* 2009), *Macrotermes subhyalinus* (39.34%) and *Macrotermes bellicosus* (39.74%) (Kinyuru *et al* 2013). All aquatic insects in this study presented an elevated level of proteins. Thus, three species in low range had less than 40% of protein, seven species fell within the range 50% to 59.50%, three species from 60% to 69%, four species from 70% to 79% and three species had over 80% protein.

Protein quality can be judged precisely in terms of its amino acid composition pattern. Nonessential amino acids can be synthesized within the body but the essential amino acid has to be solely obtained through diet. Insect body requires the same amino acid requirements as humans (Gilmour 1961). All EAAs were observed in hymenopterans with the exception of one or two depending on the species. *V. affinis* presented the whole set of EAAs while the other three were seen to be lacking in either one or two of the EAAs. The amino acid score of *V. affinis* was 96 and *P. olivaceus* was 99 and leucine was found to be the limiting amino acid in both the samples. While valine with an amino acid score of 90 in *P. varia* was found to be the limiting amino acid. The ratio between the non essential and essential amino acids in hymenopterans ranged from 1.28 - 1.68. The values of tyrosine and tryptophan in all the four samples were found to be higher and

histidine, methionine, phenylalanine, isoleucine, leucine and lysine values were lower compared to the other conventional foods as beef, pork and chicken from USDA (2015) food reference standards. Threonine level was highest in *P. varia* compared to the others.

Orthopteran insects have higher amino acid content than that of the hymenopterans. The amino acid profile compared with the FAO/WHO/UNU (1985) consultation pattern of requirement for a 2 – 5year preschool child show that *T. portentosus* is slightly deficient in methionine. Slight deficiency in threonine was seen in *C. rosea*. Threonine, methionine and lysine were consistently present in all the samples. The highest amount of total EAA was that of *O. fuscovittate* 47.89% among the orthopterans. Highest level of threonine was observed in *C. robustus.* Among the aquatic insects of order Hemiptera, Coleoptera and Odonata threonine, tryptophan and lysine were found limiting. *Nephila* sp. with the highest protein score predicted 40.82% of EAAs. In *Lepidoptera* sp. (Gunjet) tryptophan was found to be limiting. Sweet amino acids glycine and alanine and savory amino acids Aspartate and glutamate were present in the *Lepidoptera* sp. (Gunjet) though not in exact percentage proportion as in silkworm (Longvah *et al* 2011) and *Holotrichia parallela* (Yang *et al* 2014). Low level of taurine observed in *Lepidoptera* sp. (Gunjet) was consistent with tape worms, mealworms, and wild caught caterpillars and beetles however the significance of taurine in insectivores is not known (Finke 2002; Ramsay *et al* 2003). The total EAAs 66.49% in *Macrotermes sp* was higher than the other species. In order Coleoptera leucine was found to be limiting. All other amino acids detected among species of order coleoptera satisfied the recommended level (Score >100). Tryptophan and lysine were limiting amino acids in both the odonates. All orthopterans had higher protein content in comparision to the other conventional food as beef, pork and chicken from USDA (2015) food database. Although lysine content was found lower it was found to be the most consistent amino acid in all the samples. Lysine and threonine are limiting amino acids in rice and most cereals especially in India (Ozimek 1985). Consumption of the edible insect species investigated in this study in rice diet can supplement the deficiency of lysine and threonine more precisely in any cereal diets. From

nutritional perspectives it can be referred to as a source of good quality protein that has the potential to replace other conventional protein sources.

Threonine, Tyrosine and tryptophan levels of a majority of the insects were higher than that of beef, pork and chicken from USDA (2015). Tryptophan is used in treating subgroups of patients with depression, sleeplessness or hyperactive behaviors, threonine helps in maintaining protein balance Increased tyrosine intake reduces blood pressure in both normotensive and hypertensive animals (Gibson and Blass 1999). Among the non-essential amino acids glutamic acid, aspartic acid, glycine, alanine, proline, phosphoserine, serine, taurine and ornithine were the major amino acids in most of the samples. Presence of serine is important to proper functioning of the brain and central nervous system. Phosphoserine is an intermediate in the production of serine through the glycolytic pathway. Ornithine a non-essential amino acid produced in the Krebs cycle was detected in most of the insect food. Ornithine along with arginine can promote secretion of growth hormones and insulin, can heal wounds, burns and infections so low doses can be used as food supplement and high doses (above 5 g) are used as medicinal product for lowering blood ammonia concentration and in eliminating symptoms of hepatic encephalopathy associated with liver cirrhosis (Sikorska *et al* 2010). Glutamic acid, aspartic acid, glycine and alanine enhances the taste of foods by their sweet and savory taste which makes edible insects taste good. The species examined contained substantial amounts of the non-essential and essential amino acids. Studies on the amino acid profile of insects generally indicate that insects are rich source of good quality proteins and the findings of this study also pinpoints edible insect species in particular from a nutritional point of view can be regarded as a source of good quality protein or even "complete protein" in some species and can reinstate or replace other animal protein sources.

**5.1.1.4. Fats and Fatty Acids**

Protein rich foods include some amount of fats. In many instances it has been observed that foods with high protein content have lower fat content and the vice versa. In this study; similar observation was made for species having higher fat content *Ruspolia baileyi* (40.65%). *Macrotermes* sp. (39.44%) and *Lepidotera* sp.

(Gunjet) (37.13%) contained lower protein content compared to the other samples. The fat content of the species studied ranged from 4.01 – 40.65%. Fast (1970) has defined mean lipid content on dry weight basis to about 30% for larvae species and 20% for adult species. The lowest lipid content (4.01g/100g) dry weight found in *Nephila* sp. was comparable to adult beetles of *Lachnosterna* species (4.0 g/100g) dry weight (Davis 1918). Fat content in hymenopterans ranged between 15.38 – 25.53% which was higher than that of *Apis mellifera* (7.54%) (Ryan *et al* 1983). The fat content in *O. smaragdina* (22.72%), 25.53% in *V. affinis* and 19.92% in *P. olivaceus* larvae were found to be higher than that of sundried Zhejiang (15.7g/100g) and Guizhou (15.9 g/100g) ants of China (Bhulaidok *et al* 2010) and *O. smaragdina* (14.99%) in Arunachal Pradesh, India Chakraborty *et al* (2016). Fat content in *Macrotermes* sp. (39.44%) was higher than that reported for *Macrotermes Bellicosus* 36.12 % (Ekpo *et al* 2007). Fat content of orthopteras were found between ranges 11.61 – 40.65%. Coleopteran species retained adequate amounts of fats ranging between 11.76 – 20.74%. *Lepidoptera* sp. had higher fat values (33.91%) compared to *Anaphe venata* 23.22% (Ashiru 1998). Fat content among the aquatic insects ranged between 4.53 – 20.74% that was lower than that of most terrestrial insects.

Associations of SFAs through observational studies were reported to cause mortality, ischemic stroke or type 2 diabetes (De Souza *et al* 2015). However not all SFAs are hypercholesterolemic, except lauric, myristic and palmitic acids (Temme *et al* 1996). Lesser amounts of these three SFAs ranging from 0.88 -15.88% were found among the hymenopterans and it indicates less threat to human health. The most common fatty acid among the hymenopterans was 18:1 linoleic acid. MUFAs constituted the major bulk of the UFAs among the hymenopterans. *L. indicus* have higher percentages of MUFAs compared to SFAs and PUFAs which indicated SFAs and UFAs ratio of 0.42. Presence of 18:3 α-linolenic acid enriches the PUFAs content in *L. indicus.*

Among the orthopterans the PUFAs contents were found to be higher than that of beef and pork USDA (2015). Linoleic acid was found in all the species except *C. robustus, C. rosea* and *O. fuscovitatte*. Linolenic acid is accounted for its

major influence over the clotting mechanism, as well as stabilizing the heart against arrhythmia that can lead to sudden death (Hayes 2002). Presence of linolenic acid boasts the fatty acid profile of the studied species which indicates its importance in nutrition. High fat content indicates high calories in food which is particularly desirable for people carry out much manual work. Even though the fat content of the edible insects were low, this study indicated higher UFAs levels in which makes it a desirable food. Presence of both saturated (SFA) and unsaturated fatty acids helps in the assessment of the quality of a fat though complex the (UFA) in these insects could be an advantage since they may complement each other's physiological functions (Chakraborty *et al* 2016). Higher SFAs in any food is undesirable due to its linkage to atherosclerotic disorders (Reiser, 1973). PUFA composition of most of the species proved to be higher and superior to that of beef and pork. Therefore, the study indicates use of edible insects in food formulation towards better nutrition and human health.

**5.1.1.5 Calorific Values**

The different fat contents of the edible insects are a reflection of their calculated gross energy values of ranging from (410.7 – 580.25 kcal/100 g) which lie within the range of those that of Ramos-Elorduy *et al* (1997) reported for seventy eight species of edible insects. Although the energy values were comparable to those of other edible insect species as well as calorific values for rice (345 kcal), wheat (345 kcal), and egg (173 kcal) (Srilakhsmi 2012) none of the species could meet the recommended dietary allowance which is 2500–3500 kcal/100g the recommended allowance for energy. However, this gap can be fulfilled when insect foods are ingested along with other food items.

**5.1.1.6 Carbohydrate, Starch and Dietary fibres**

Carbohydrate content calculated out from the proximate ranged between (0.48 – 37.78%) and was comparable to those described by Ramos Elorduy *et al* (2011) for twenty-five edible orthopterans in Mexico. Crude fibre and starch components were appreciably lower. Differences in detecting nutrient composition could arise due to different analytical methods used or diverse states of health of the examined specimens or due to varied habitats they came from.

## 5.2 Biochemical evaluation

### 5.2.1 Growth performance of rats

Least amount of feed consumption was observed in animals fed with protein free control diet. The animals consuming the test diet containing 10 % crude protein (N x 6.25) and reference diet containing 10% standard casein showed no differences in feed intake. Weight gain is a measure of growth so the animals fed on basal diet showed apparent growth failures. The group of rats fed *C. tripunctatus* showed better growth performance during the fourteen and twenty-eight days feeding periods and recorded the highest weight gain. The feed intake and weight gain in test diet were found to be slightly lower in the course of feeding period for 14 days and much lower during the course of 28 days feeding periods compared to the reference diet. Reduced feed intake can be attributed to factors as imbalance of antinutrients or reduced palatability in the diet. Feeding processes influences the nutritional value of feedstuffs by enhancing or lowering them (Ensminger 1985) the observed feed intake differences may be due to the fat content that adds flavour to the food and improves dietary intake. The PER value for all the samples were found to be lower than the value obtained for the casein diet and this result is in agreement to Calvert *et al* (1971). Insects protein qualities were proved to be superior from various protein sources as soybean and casein. A young animal continuously increases its food intake during its rapid growing period. The gain of weight per gm protein consumed during the 14-day period was found to be slightly higher than that for 28 days. This result coincides with the result of Osborne *et al.* (1919) where slightly higher values were recorded in animals fed protein diet for four weeks compared to 11 weeks, the reason explained was that the former four weeks were the rapid growing periods for the animals so higher amount of feed intake and growth rate was observed. The PER (Protein Efficiency Ratio) values of all the samples were slightly lower than that of standard casein but were not significant at ($P < 0.05$). Significant lower amount of PER was observed in *L. indicus* and *O. smaragdina* towards the 28 days feeding period. The PER values showed that lower feed intake resulted to lower PER values. Higher values of PER were observed for samples as *R. Baleyi* with lower total protein concentrations. Higher PER values represents good quality proteins Osborne *et al.* (1919). This is evidence to the fact that protein

quality cannot be judge in terms of its total protein content. The Net Protein Ratio (NPR) values of rats fed for 14-day diet were slightly higher but were not significant ($P < 0.05$) from control. High biological values (BV) were observed for the studied species though significantly ($P < 0.001$) higher values were obtained for casein. These results confirm that maximal utilization of good quality proteins can occur even at lower amounts of protein intake while higher tendencies for increased metabolic wastage of dietary proteins may occur in higher protein intakes (Ekpo 2011). The ease of bioavailability of amino acids in nutrition is an important factor for measuring the protein quality, which is indicated by the values of True Digestibility (TD). TD values as high as 89.20 was observed by Ekpo (2011). Higher TD values were found for rats fed the test diet for 28 days. However, looking at the proximate and chemical analysis testimonial for the test diets and judging against these with the observed weight gain, Protein efficiency ratio, Biological value, True digestibility and other nutritional parameters, does not imitate the experimental values. Generally, the organs of the animals placed on the protein free basal diet were pale in appearance and lighter in weight as compared with those of the other animals placed on the standard casein diet and test diets. The liver and kidneys of the animals placed on the casein diet appeared normal with no lesions on them. No differences were observed in animals fed the test diet and the reference diet.

### 5.2.2 Organ Weights

The results of the organ weight clearly indicate that there was corresponding increase or decrease in the organ weights compared with the protein intake. Significant increases and decreases in relative organ weights were observed in all the animals from the control. A direct correlation existed between feed intake and relative liver weights of the test animals. Feeding low quality proteins for a long period of times affects the organ weights and liver specifically Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes (Kimiagar 1979). Nevertheless, it was reported by Harizal *et al* (2010) that body weight increases of animals are more closely related to the accumulation of body fats rather than exposures to toxic effects. The ratio of the relative weight of spleen to the body

weight remains fairly consistent regardless of age and in rats the value typically ranges around 0.2% (Losco 1992). The significant decrease ($P < 0.05$) seen in the spleen weight of *C. tripunctatus* fed diet from the control towards the 28 days feeding period can be considered as normal with no adverse effects of toxicity.

**5.2.3 Hepatic indices**

Liver is involved in different aspects of carbohydrate metabolism, lipid metabolism, protein metabolism and xenobiotic metabolism. Deviations from these normal metabolic functions are indications of stress. Different enzymes as transaminases and phoaphatases are involved in the catalysis of reactions involved in these metabolisms. Enzymes as ALT and AST are transaminases that catalyses the transfer of alanine and aspartate to glutamate. GGT is involved in the transfer of gamma-glutamyl functional groups and ALP are phoaphatases that hydrolyses phosphates. Several factors can induce the secretion of these enzymes. Elevated or decreased levels of these serum enzymes serve as indicators of stress. A significant decrease in ALT activity and a significant increase in ALP activity from the control was observed in *C. tripunctatus* diet fed animals but the observed values were within the reference ranges (45 - 135 $UL^{-1}$) for ALP and (5 – 45 $UL^{-1}$) for GGT assigned in the test kits. A raised GGT in the presence of a raised ALP indicates that the liver is the primary source of the resulting causes (Thomas *et al* 1998; Moss and Henderson 1999; Tietz *et al* 1983; Burtis and Ashwood 1999). The concentration of AST was found to be higher than the ALT levels in all the animals which were in accordance to Mayne (1996) who described that body cells contain more AST than ALT. ALT is an important liver enzyme in rats, rabbits, cats, dogs and primates and is a sensitive marker for hepatocellular disease that can provide a quantitative measure of liver damage compared along with the levels of AST (Farah *et al* 2011; Al-Mamary *et al* 2002). A significant ($P < 0.05$) increase in the level of these enzymes was seen in the basal diet fed protein free group which may be an indication to macroscopic histopathological changes. The ALT, ALP, AST and GGT levels were normal in all the rats fed the test diets for 14 as well as 28 days. This result was also confirmed by the absence of histopathological changes in the liver. Hence, the observed differences and apparent lack of any effects of any of dietary intake on

these enzymes makes it difficult to ascertain any disease condition suggesting that the four test diets or edible insects consumption causes no hepatotoxicity.

**5.2.4 Renal indices**

A significant decrease ($P < 0.01$) observed in the serum protein concentration of *C. tripunctatus* and a significant increase ($P < 0.05$) observed in the serum protein concentration, urea as well as Blood Urea Nitrogen (BUN) of *L. indicus* in comparison to the control towards the 14 day as well as the 28 days feeding periods is indicative of abnormalities in kidney dysfunctions. The kidney along with the liver plays vital role in detoxification. Urea measurements in blood are performed to evaluate normal kidney functions. According to (Miller and Payne 1961) if energy concentration in the diet is too low, some of the protein gets deaminated inevitably and is used up for energy. Since lower feed intakes of it was found in *L. indicus* the low energy level may have been one of the factors contributing to the higher levels of blood urea nitrogen. The plasma total protein and blood urea nitrogen levels reflect the quality of the protein sources. The higher concentration of blood urea indicates the presence of large quantities of amino nitrogen resulting from the breakdown of tissue or dietary proteins or both (Ekpo 2011). The renal indices of *R. baleyi* and *O. smaragdina* were not significant ($P > 0.05$) in comparison to the normal and this gives a clear indication that these diets have no toxic effects in the renal functions. The Urea and BUN values were all within the reference range 10 – 50 mg/dL for Urea and 5 – 23 mg/dL for BUN so consumption of the four edible insects induces null effects on the renal indices of the consumers.

**5.2.5 Serum Cholesterols**

No significant ($P < 0.05$) increase were observed in the serum cholesterol levels of the animals fed the test diets for 14 and 28 days except a significant ($P < 0.05$) increase in High Density Lipoprotein (HDL) levels in *O. smaragdina* fed diet for 28 days were observed which may be due to the presence linoleneic acid in this sample. Similar results were obtained by Longvah *et al* (2012) through experiments of feeding edible oils of Eri silkworm to rats. The total cholesterol, triglycerides and Low Density Lipoprotein (LDL) were found to be within the reference ranges for total serum proteins 6 – 8 $gdL^{-1}$, total cholesterol <200 mg $dL^{-1}$ triglycerides 40 –

165 mg $dL^{-1}$, HDL >55 mg $dL^{-1}$ and LDL <150 mg $dL^{-1}$ assigned in the test kit. Even though a significant increase in the levels of HDL in the course of the feeding periods were detected the resultant values were lower to the reference range for HDL >55 so it is suggestible that consumption of *O. smaragdina* larvae for a longer period can elevate HDL levels in blood. Higher values of triglycerides are linked to atherosclerosis and increase the risk of heart diseases. Increased HDL on the contrary to total cholesterol, serum triglycerides and LDL may reduce the death risk due to heart diseases. It is always imperative to emphasize the reason of cholesterol elevations in studies because this may attribute to blockage of liver bile ducts leading to choleatasis (Rai *et al* 2009).

**5.2.6 Haematological indices**

Haematological indices are the most susceptible target of toxic compounds and blood parameters are the indicators relevant for risk evaluation. It represents an important index for evaluating the physiological and pathological status in humans and animals (Hazilawati et al 2007). Consumption of the test diet and the control casein diet caused no abnormalities in haematological indices. The Pack Cell Volume (PCV) value of *L. indicus* and *O. smaragdina* were significant ($P < 0.05$) to the control after the 14 days feeding regime but no significant differences in PCV values were observed towards the 28 days feeding in this two sample indicating no loss or destruction of red blood cells. *L. indicus* showed extremely significant ($P < 0.05$) increase in haemoglobin (Hb) concentrations which may be due to its high iron values. No significant differences were observed in the White Blood Cells (WBC) and Red Blood Cell (RBC) values in comparison to the control towards the 28 days feeding period. However; small reductions in the numbers of WBC and platelet counts were observed in the 28-day test diet fed animals compared to the 14 days test diet fed rats. However, reductions in WBC (leukopenia), RBC (anaemia) and platelets (thrombocytopenia) not accompanied by signs of clinical toxicity as severe diarrhea, blindness and mortality are usually linked to bone marrow suppression (Knight and Walter 2001). The RBC indices Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentrations (MCHC) of the animals on the reference and insect diets were in the

same pattern and did not reveal significance to the control. The RBC indices observed below normal range is indicative of different types of anaemia (Goljan 2014). The similarities in the findings for the test samples suggest that the animals were capable of utilizing these samples with almost equal efficiency.

**5.2.7 Histological indices**

Histological observations revealed no evidences for toxicity. Liver gets exposed to xenobiotic-induced injury because of its central role in xenobiotic metabolism and its portal location within the circulatory system (Jones 1996). Severe hepatic injuries as in severe necrosis the liver enzymes are released into the blood. Liver necrosis is an important clinical conditions of liver in which is a complex process where simultaneous activation of multiple deregulated pathways culminates to the loss of cell membrane integrity causing leakage of cellular constituents (Trey and Davidson 1970). Fatty changes in liver are associated with metabolic disturbances and toxicity which may result into lesions. Histological studies of the liver of animals fed the test and control diet in the present toxicity study showed no signs of lesions, necrosis and infiltrations. Protein casts observed in the renal tubules through microscopic examination of the renal tissues are characteristic of renal toxicity (Hazilawati *et al* 2009). Other prominent microscopic characteristics of renal toxicity include interstitial fibrosis, dilatation of the renal tubules and chronic inflammation. The observed glomerular tufts were not contracted or vacuolated and the renal tubules appeared normal coated by a single layer of densely stained cuboid cells. No characteristic changes indicating renal toxicity were observed in any of the animals fed the different diets. Hence the histology study revealed no signs of renal toxicity. Even the renal function of the animals fed the protein free basal diet appeared normal. The testicular sections were examined for effects or any kind of toxicity that may result to infertility due to the feeding of test diets. Degenerative tubules, evaluation of the germ cell and sertoli cell ratio and the number of leydig cells, the presence or absence of seminiferous tubule hyalinization, and presence of other findings such as tubular cells with carcinoma, interstitial inflammation, or granuloma are some of the common indicators of male infertility (Abdullah and Bondagji 2011). The present histological

study presented testicular sections with normal spermatogenesis showing orderly progression from spermatogonia to spermatocytes encompassing groups of spermatids and mature spermatozoa. Degenerative lesions such as atrophy and fibrosis, lipidosis as fatty infiltration, splenic necrosis, lymphoid necrosis or apoptosis, vacuolization, focal red pulp hyperplasia, focal white pulp hyperplasia, granulocytic erythro and mononuclear leukemias, Sarcomas, Mesothelioma and other tumours are the abnormalities that can be detected through histological spleen examinations (Suttie 2006). No findings of such abnormalities were observed in the spleen architect of the microscopically examined spleen sections which indicated the normal functioning of the spleen. Therefore, it is evident from the findings in this study that the consumption of the four edible insects is not toxic to the liver, kidney, spleen and testis. Similar results on the toxicity evaluation were also observed by Longvah *et al* (2012) after the feeding of Eri silkworm pupae oils to albino rats even for a long period of 12 weeks. In conclusion; this study confirms that edible insects do not possess toxicity as indicated in our rat model. No deaths or signs of toxicity were observed in the rats thus establishing its safety for consumption in the diets of the Bodos in selected areas of Assam, Northeast India as well as other consumers worldwide.